CHANGES IN INNATE IMMUNE PARAMETERS AND GUT PHYSIOLOGY IN RAINBOW TROUT (*Oncorhynchus mykiss*) SUBJECTED TO FEED RESTRICTION AND REFEEDING

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Introduction

Wild fish can often withstand periods of food restriction or deprivation due to seasonal fluctuations in environment conditions and food availability or during the breeding season. In captivity this situation could be simulated by the farmer to exploit compensatory growth during the re-feeding period in order to reduce feed costs and optimize growth performance. The effect of starvation or feed restriction and subsequent re-feeding in different fish species have been the subject of a number of studies mainly focused on somatic growth, metabolic and endocrine adaptive responses. The current study was aimed at investigating the response to a 3-week fasting/restricted feeding period on welfare state and gut condition of rainbow trout (*O. mykiss*) throughout the following 2 weeks of re-feeding. To this end non-specific immune defense parameters, the activity of intestinal brush border membrane enzymes and the composition of the gut microbiota were investigated.

Material and methods

Ninety-nine trout (body weight 109.1 ± 3.5 g) were randomly distributed among 3 tanks, each of 0.5 m^3 capacity and supplied with 8 L min^{-1} of well water at a temperature of 12.7 ± 0.8°C. The fish were subjected over 5 weeks to one of the following treatments: C, control, continuous feeding with a commercial trout feed (45% crude protein, 28% crude lipid) at 1.3 % BW over 5 weeks; R, a restricted ration (30% of C) over three weeks followed by two weeks re-feeding to visual satiety; F, fasting over 3 weeks followed by two weeks re-feeding to visual satiety. At the end of the 3 weeks fasting/restriction period (T0) and after 7 (T7) and 14 days (T14) of re-feeding, 3 fish per treatment were netted and anaesthetized with 0.1g/l of MS-222 and blood was withdrawn from caudal vessels to be used for analyses of plasma lysozyme, perossidase and antiprotease activity as described by Bulfon et al., (2017). The same fish were then euthanized with a lethal dose of anaesthetic and sampled for analyses of intestinal brush border enzymes activities (maltase, sucrase, intestinal alkaline phosphatase (IAP) and γ-glutamil transaminase (γ-GT)) (Harpaz et al., 2005) and for fish gut microbiota profiling through NGS technology. In particular, DNA was extracted using the standard protocol for the ZR-96 kit, with bead beating with a Genogrinder high-throughput tissue homogenizer (SPEX SamplePrep, Metuchen, NJ) and stored at –20°C until further processing. Amplification and sequencing were performed as described previously for bacterial communities (Bokulich et al., 2012). Data analysis was performed as described by Bokulich et al. (2013).

Results

Non-specific immune response parameters. At the end of the 3 weeks fasting/feed restriction period (T0) no significant differences were found among treatments in lysozyme and perossidase activities ($P>0.05$), while the antiproteases was found significantly higher in fasted fish ($P<0.05$). No significant among treatments changes in the same parameters were observed after 7 or 14 days of re-feeding.
Intestinal BBM enzyme activities. When compared to continuously fed fish, those subjected to a feed restriction or starved over 3 weeks resulted in significantly diminished activity of maltase, sucrase and IAP. After 7 days of re-feeding no differences were observed among groups for the gut enzymes considered, while after 14 days the pattern of

Table I. Specific activity of the brush border enzymes in the test groups at sampling times.

Sampling time (days)	Treatment	Maltase U/mg prot	Sucrase U/mg prot	IAP mU/mg prot	γ-GT mU/mgprot
0	Control	11,01±2,24[a]	5,08±1,36[a]	508,9±135,52[a]	4,43±2,90
0	Restricted	11,18±3,95[a]	3,34±0,24[b]	468,9±207,19[ab]	2,56±1,51
0	Fasted	5,05±2,49[b]	3,12±0,67[b]	311,9±110,98[b]	2,38±0,75
7	Control	16,65±5,33	4,57±2,23	525,8±167,52	7,54±3,99
7	Restricted	14,72±1,72	4,92±2,12	487,6±185,52	8,42±6,47
7	Fasted	11,18±8,75	5,28±1,71	468,7±104,65	8,38±5,78
14	Control	11,73±3,32[b]	3,69±0,99[ab]	485,6±130,60[b]	3,95±3,19[b]
14	Restricted	17,21±2,87[a]	4,45±1,25[a]	1135,9±411,21[a]	5,21±3,77[b]
14	Fasted	16,36±2,80[a]	2,27±1,51[b]	494,5±63,93[b]	10,35±3,96[a]

the activity of the intestinal brush border enzymes varied among the different treatment. In particular, the activity of γ-GT was significantly increased in previously fasted fish.

Gut microbiota profile. At the end of the fasting/restricting period, in R and F groups the Actinobacteria phylum was partially substituted by Bacterioidetes and Firmicutes ones; in addition, other unknown phyla and cells from the Eukariota kingdom where observed. After 7 and 14 days of refeeding the major phyla considered were substantially similar to that of the control group.

Discussion

The aim of the present study was to ascertain to what extent rainbow trout got homeostatic recovery when liberally re-fed after being subjected to a substantial feed shortage or deprivation. Our results have shown that 3 weeks of fasting or feed restriction did not significantly impair certain innate immunological parameters; moreover, the high level of the anti-protease activity observed in F group at T0, suggests that fish kept starved could even enhance certain defence mechanisms. Whereas in previous study (Martin et al., 2010) fasted fish have generally decreased transcription of immune genes, our data suggest that this does not necessarily implies changes in the level of plasma proteins.

In fasted fish the lack of the substrates has depressed the activity of intestinal disaccharases after three weeks of treatment, while the significant increase after 14 days of refeeding indicated a full recovery of the capability of nutrient utilization. The IAP enzyme is considered a marker of the differentiation and maturation of the enterocyte and 3 weeks fasting had significantly decreased its activity in the fasted fish. The γ-GT is involved in aminoacid transport in the gut thus its increased activity during the refeeding period is consistent with the increasing protein availability for the digestion.

Our results also demonstrate that a feed restriction and fasting directly affect trout microbial community and refeeding rapidly shift and restore the gut microbiota phyla profile.

The knowledge gathered from this preliminary study will be a useful tool to optimize fish feeding management to exploit compensatory growth without affecting fish welfare.

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